HAEMOGLOBIN POLYMORPHISM IN ASIAN ELEPHANT *ELEPHAS MAXIMUS* WITH SPECIAL REFERENCE TO ELEPHANT POPULATION IN SOUTH INDIA[1]

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(With a text-figure)

Polyacryl Amide Gel Electrophoresis (PAGE) was used to study haemoglobin patterns in Indian elephants *Elephas maximus*. Blood samples were collected from 12 captive elephants at Mudumalai Sanctuary, Tamil Nadu. Out of 12 elephants, 10 (83.33%) showed two band Hb profile whereas 2 individuals (16.66%) showed a solitary haemoglobin (Hb) band, suggesting homozygosity of gene expression.

INTRODUCTION

Biochemical differentiation of species specific proteins can be established by using electrophoretic techniques (Selander *et al.* 1969, Yoshida *et al.* 1971 and De Smet and William 1978). Ferguson (1980) had recognized that although this technique was useful in establishing differences between forms, it was not successful in bringing out similarities between them. The limitations of the technique are overcome in the comparative blood sample study of various forms collected in an identical set of conditions with appropriate controls and the objective analysis of the data. Hb, being a stable molecule, is very useful in biochemical studies. There is no record of Hb patterns in elephants in available literature. P.E.P. Deraniyagala (1955) has proposed 7 living subspecies of the Asian elephant *Elephas maximus*, of which two are from India. This classification was based on morphological differences, amount of depigmentation and proportion of tuskers to tuskless males (*Maknas*) and tushed males (*Aliyas*).

Further, he has suggested that the Asian elephant is polymorphic and its wide range of individual variation has obscured the existence of a number of subspecies which have been revealed by studying the intensity with which certain variations are localized to different areas. In Sri Lanka and India, 10 main varieties and numerous sub-varieties have been recognized from the earliest times, subdivisions being based on shape, size, colour, voice, behaviour, strength, body odour, diet and susceptibility to certain diseases. In the light of this information it was felt that the use of PAGE may help in establishing biochemical polymorphic pattern, if any, in the Indian elephants. The frequency of occurrence of polymorphic locii and possible genetic inter-relations may also be ascertained by the analysis of the data on PAGE.

We are also working on 3 other proteins, viz. LDH, Esterase and SDH to assess polymorphism at specific gene locii. The results of these studies will be published in due course. We also plan to collect blood samples from the elephant populations of central and northern India to find out genetic affinities or differences, which will help in suggesting sub-specific status of the populations proposed earlier by different authors.

MATERIAL AND METHODS

Blood samples of 12 elephants were collected by Dr. V. Krishnamurthy, Project Officer, Indian Elephant Project, BNHS. Blood was withdrawn from the veins on the back of the ear using sterilized syringes and 16 gauge needles. Samples (5-10 ml each) were transferred to sterilized PVC bottles and allowed to thaw at room temperature for a few minutes to separate the serum. Then all the bottles were transferred to a liquid nitrogen cylinder(LN_2) to freeze them at–192°C. The samples were then transported with the cylinder to R.J. College, Bombay for laboratory analysis. Hb was solubilised from isolated RBC's after a treatment with hypotonic saline (0.02%).

Haemoglobins were separated by PAGE under precisely controlled factors like gel concentration (7.5%), buffer system (Tris-glycine, pH 8.4), voltage 250 mV, current 4 mA per tube, ambient temperature (4°C ± 1° C), duration of run etc. Some of the samples were run successively to check the constancy of all factors which helped in the analysis

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TABLE 1
HAEMOGLOBIN VARIANTS IN TERMS OF RM VALUES, THEIR OCCURRENCE AND FREQUENCIES IN A SMALL DOMESTICATED ELEPHANT POPULATION IN SOUTH INDIA

Rm values with S.D.	No. of bands	% population (occurrence)	% frequency of bands
0.345 ± 0.015	1	8.33	4.54
0.355 ± 0.005	2	16.66	9.09
0.355 ± 0.015	1	8.33	4.54
0.37 ± 0.01	1	8.33	4.54
0.37 ± 0.02	1	8.33	4.54
0.375 ± 0.005	1	8.33	4.54
0.38 ± 0.02	1	8.33	4.54
0.385 ± 0.025	1	8.33	4.54
0.395 ± 0.005	1	8.33	4.54
0.56 ± 0.01	1	8.33	4.54
0.57 ± 0.02	1	8.33	4.54
0.575 ± 0.015	2	16.66	9.09
0.59 ± 0.02	1	8.33	4.54
0.595 ± 0.015	2	16.66	9.09
0.605 ± 0.015	3	25.00	13.63
0.61 ± 0.01	1	8.33	4.54
0.615 ± 0.015	1	8.33	4.54

Total no. of specimens – 12 Total no. of bands– 22

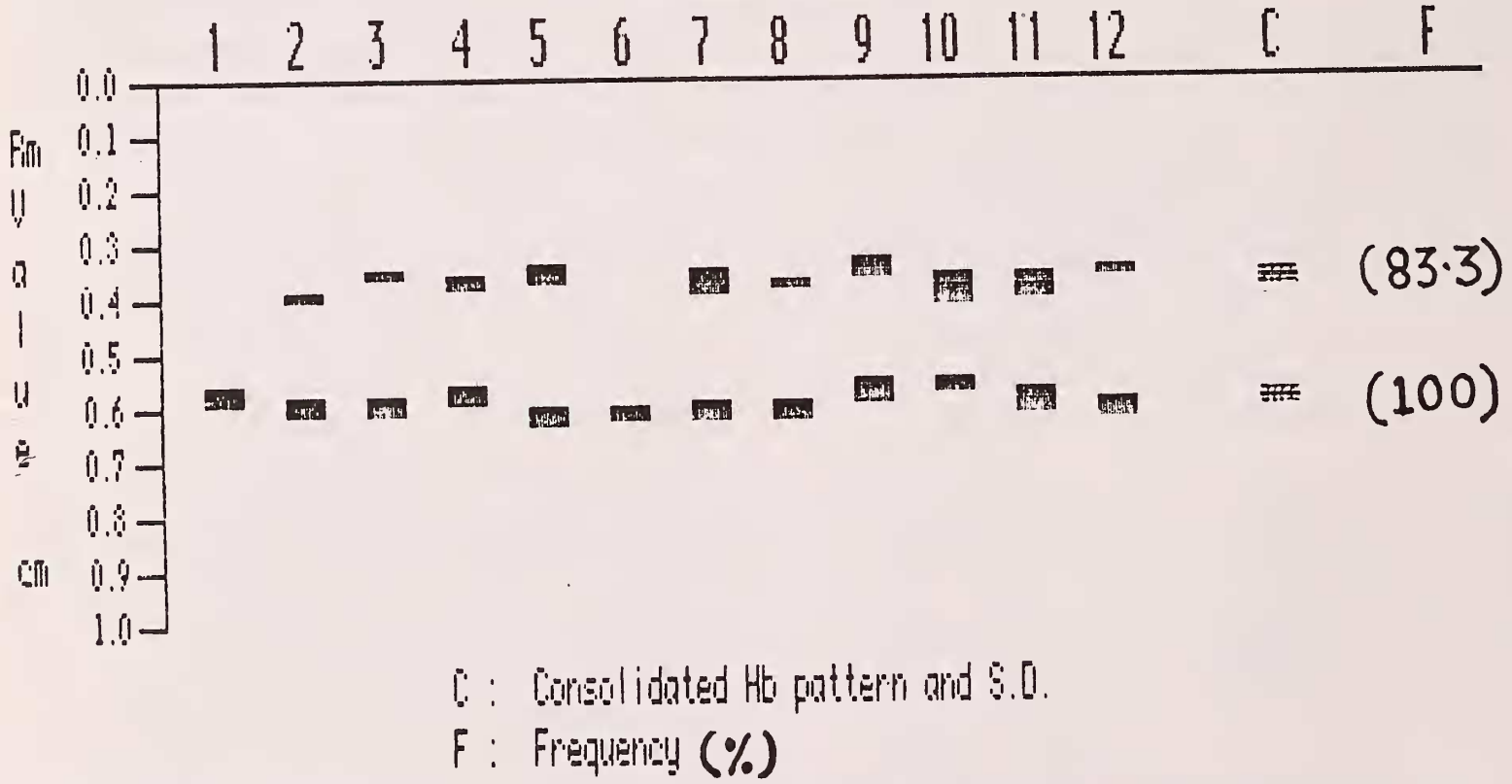

Fig. 1. Diagrammatic representation of haemoglobin profile of a small domesticated elephant population in south India

of the results. The dye Bromophenol Blue was loaded on the gel columns set in neutral glass tubes along with samples and served as a marker. For staining the gels after electrophoresis to identify Hb fractions, we followed the procedure of Ornstein (1967). Relative mobility (Rm) of Hb was calculated as a ratio of the distance travelled by Hb from the base to the distance travelled by the marker in the same run. The mean Rm with a standard deviation from the mean for each identified Hb band was recorded. Hb profiles for each individual were prepared by plotting Rm values and a consolidated

Hb profile for the population was constructed. Percentage occurrence of a fraction in the population and percentage frequency of each band was calculated. The band of the highest mobility was numbered Hb1 and band of lower mobility was numbered Hb2 as suggested by Ferguson (1980).

Results and Discussion

Table 1 shows records of the relative mobilities of haemoglobin fractions for 12 individual. The number of occurrences of a band in the population and its percentage frequency is given against each band. The same data are represented in Fig. 1 as a consolidated diagrammatic profile for the samples studied.

It is clear from Fig. 1 that out of 12 individual, 10 showed 2 pattern and 2 individual showed a solitary band suggesting homozygosity of gene expression for the Hb_1 locii. Thus it is evident that 16.66% of the population have homozygous expression, whereas 83.33% population show two banded expression. The Hb_1 pattern with the highest mobility is strongly expressed as compared to Hb_2 pattern. A single band profile for any protein is considered as an expression of homozygous gene locii specific for such protein (Ferguson 1980). However in the present study a homozygous pattern was found only for the locii of Hb_1 and not Hb_2. However, it is likely that the Hb_2 is also an expression of an independent gene locii with a slightly less frequency in the population.

Polymorphic expression of gene locii for Hb has been reported for rodents (Pradhan *et al.* 1984, Selander *et al.* 1969), birds (Sane *et al.* 1986). The Hb profile with an individual variation of single band to as many as a total of 15 bands on consolidation are reported for rodents, whereas for birds the number varies from one to six on consolidation. Thus in comparison, a two band pattern on consolidation in elephants appear to be more conservative.

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